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Cytokines, Chaos, and Complexity

Review

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One of the biggest challenges facing biologists today is how to integrate the many components that make up a cell, organism, or ecological community in a way that helps us to understand the function of the whole. This problem has become even more acute with the successes of the human genome project, which hopes to identify every human gene within the next 1–2 years. Nowhere is this complexity better illustrated than in the cytokine network. Cytokines are small protein or glycoprotein messenger molecules that convey information from one cell to another. Most are secreted but some can be expressed on the cell surface or held in reservoirs in the extracellular matrix. More than 200 have now been identified including the interleukins, growth factors, chemokines, interferons, and a host of others. Most if not every cell in the body both produces and responds to cytokines of one sort or another. Amino acid sequence and structural comparisons have shown that cytokines can be grouped into at least six different families. The biggest of these is the family of four α helix bundle cytokines typified by interleukin 2 (IL-2). All cytokines bind to specific receptors expressed on the surface of the target cell, thereby triggering complex intracellular signaling cascades, which ultimately control gene expression required for the cellular response. Many of the receptors have also been cloned and their molecular structures elucidated. The receptors can also be grouped into families based on their structural similarities.

When they were first discovered less than twenty years ago it was believed (and hoped) that each cytokine would convey a unique signal for a defined cellular response. This would have made them easy to understand because each cytokine would be defined by the response it evoked. It did not take long to appreciate how wrong this concept was (Figure 1). It is now known that most cytokines have multiple and diverse biological functions. For example, fibroblast growth factor (FGF) is involved in wound healing and embryonic bone development. IL-4, on the other hand, is a key cytokine in T cell differentiation, IgE production, and endothelial cell activation. Many cytokines are produced by more than one cell type and act on a variety of target cells at different stages of cellular proliferation and differentiation. In addition, most cells produce many different cytokines.

The production of cytokines and expression of cytokine receptors is under tight but complex biological control, including negative and positive feedback by the cytokines themselves. In many instances the outcome of cytokine signaling is modified by other cell surface receptor ligand interactions. The relationship between cytokines and their specific receptors is also very complex. In most cases, cytokine receptors are made up of two or more different subunits that may be shared with receptors for other cytokines. For example, the γ chain of the IL-2 receptor is also a component of the receptors for IL-4, IL-7, IL-9, and IL-15. Many cytokines bind to more than one receptor, e.g., IL-1, TNF, and IL-4, which can have quite distinct patterns of expression and signaling functions. The resulting system is a network of such staggering complexity that it is a wonder it does not collapse into complete disarray. Remarkably, it can even remain stable when key cytokines are removed from the network in gene inactivation experiments.

Although many details of particular cytokine interactions have been elucidated and the effects of cytokines on a myriad of cellular functions have been described (more than 12,000 papers on cytokines have been published in the last year), practically nothing is known about the behavior of the network as a whole. This is particularly evident by the lack of progress in understanding complex diseases such as rheumatoid arthritis or allergic asthma by measuring cytokine levels (Woo, 1997; Barnes and Marsh, 1998) and by the almost universal failure to predict the outcome of cytokine gene inactivation experiments, for example, LIF (Stewart et al., 1992) and IL-2 (Schorle et al., 1991). Perhaps the most important features of the network are nonlinearities in cytokine interactions and the presence of positive and negative feedback. Complex nonlinear systems commonly have unusual and nonintuitive properties that may include chaotic behavior. These properties make the cytokine network too complex to be understood fully by the conventional experimental approach of testing the effects of cytokines or combinations of cytokines on cells in vitro. A complementary modeling approach based on modern nonlinear dynamic is now required.

What Is a Nonlinear Interaction?

In simple terms, nonlinear interactions are those in which the output is not proportionally related to the input. All cytokine interactions exhibit nonlinear behavior. Typical examples are cytokine dose-response curves showing

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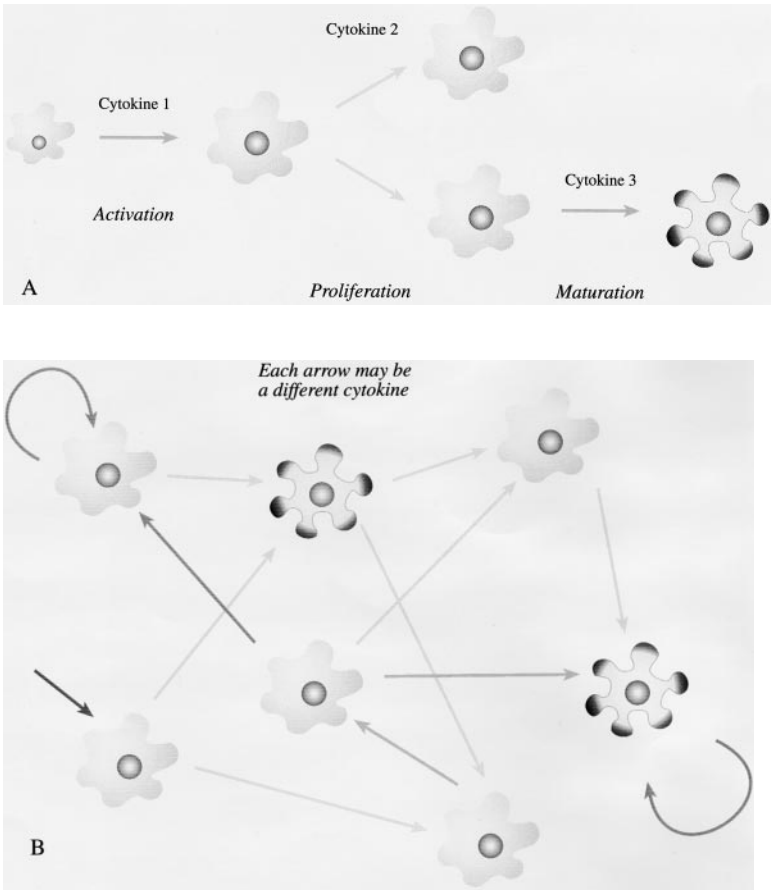

Figure 1. Linear and Network Models of Cytokine Action

Linear models of cytokine control of cellular function (A) have been replaced by network models (B) that take into account cytokine interactions.

no effect below certain concentrations followed by an exponential increase in response with a plateau maximum response or even reduced response with high cytokine concentrations (Figure 2). An excellent example of nonlinearities in cytokine responses was reported recently for genetic control of TNF production (Amiot et al., 1997). In these experiments, TNFα secretion in response to stimulation with bacterial lipoploysaccharide (LPS) was compared in wild-type mice (TNFα$^{+/+}$), mice in which one allele coding for the *TNF*α and lymphotoxin (*LT*α) genes (both are found in tandem on mouse chromosome 17) was deleted (TNFα$^{+/-}$), and mice in which both alleles had been deleted (TNFα$^{-/-}$). As expected, high levels of TNFα were detected in the serum of wild-type mice after in vivo administration of LPS, whereas no TNFα was detected in the homozygous −/− mice. Surprisingly, very low levels (60 times lower) of TNFα were detected in heterozygous +/− mice following treatment with LPS, with a corresponding effect on LPS-induced mortality. As the gene dosage was one half that of the wild-type +/+ mice, the much lower response of the heterozygous mice indicated a nonlinear effect. It was found that the difference between TNFα production by monocytes from wild-type +/+ and heterozygous +/− mice increased with higher concentrations of LPS and over time, from about 2-fold at 4 hr to 20- to 100-fold after 18 hr; a characteristic of a positive feedback loop. Such nonlinear cytokine interactions have important implications for understanding the role of cytokines in disease. It is particularly relevant for interpreting the recently described associations between cytokine and cytokine receptor gene polymorphisms and immune function in disease (Mullighan et al., 1997; Turner et al., 1997; Hobbs et al., 1998; Hurme et al., 1998).

Nonlinear interactions of the sort described above are familiar to most cytokine biologists, but their implications for the behavior of the cytokine network may not be generally understood. Nonlinear interactions can give rise to effects even in quite simple systems that are often counterintuitive, including the unusual properties of chaos. In more complex systems such as the cytokine network, nonlinear interactions between the different components may give rise to rather unexpected behavior that is not always recognized or taken into account.

Cytokines and Chaos

One of the most important features of nonlinear systems is that small changes in initial conditions can be rapidly amplified to magnitudes comparable to the full dynamic range of the system. Mathematicians refer to this phenomenon as “chaos” (or more precisely such sensitive dependence is one of the main defining characteristics of chaotic systems). Chaotic behavior can bring both advantages and disadvantages. On one hand, if we understand a chaotic system well enough, we may be able to control it using only very small control actions. This

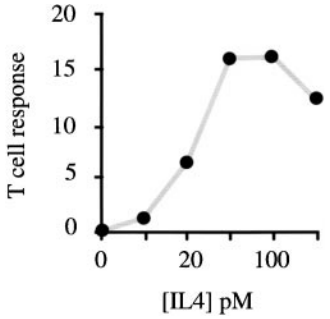

Figure 2. Typical IL-4 Dose-Response Curves for T Cell Responses

has already been demonstrated in a wide range of experimental systems ranging from lasers to cardiac tissue (Shinbrot et al., 1993). On the other hand, sensitive dependence on initial conditions can make the long-term behavior of a system difficult to understand and impossible to predict. This does not mean, though, that the behavior is random or nondeterministic. These features can be illustrated with the classic example of the motion of the planets and other celestial bodies under Newton's laws of motion. This in fact was the system in which chaos was first discovered by Poincaré some 100 years ago and is known as the three body problem. He showed that if one took just three celestial bodies whose interactions are governed by Newton's laws of motion and let their positions evolve under the influence of gravity, their behavior could be so complex as to defy complete understanding. In this system, immeasurably small variations in the starting conditions of one or more of the three bodies may be rapidly amplified and after a short time give rise to completely different trajectories. Since we can never measure the initial position of any celestial body (or cytokine concentration for that matter) to arbitrary accuracy, this means that the long-term behavior of the system is essentially unpredictable, even though it is deterministic. This is despite the fact that we only have three interacting bodies and the interactions are just Newton's law of motion ($F = ma$) combined with the inverse square law of gravitational attraction (which provides the nonlinearity in this case).

At first sight, chaotic behavior of this kind may seem to be far removed from the functioning of cytokine networks. These after all have a fundamental role to play in many of the organism's functions and hence cannot afford to be prone to every tiny perturbation. It has to be stressed, however, that although individual trajectories in a chaotic system may be very sensitive, the overall statistical properties of a chaotic system can be quite robust. It is thus possible for a system to be chaotic and still perform a useful function. Furthermore, there can be significant advantages to operating in a regime where nonlinearities give rise to sensitive dependence on initial conditions, as small properly timed interventions can give rise to a large but desired effect. In the context of cytokine networks, this approach may enable therapies to be designed that move the network from one state to another (say from an inflammatory response to an noninflammatory one) by using small carefully controlled interventions, rather than brute force. Of course, in order to have any hope of doing this, one needs to understand the dynamics of the whole network far better than we do today.

Complex Dynamics in a Simple Cytokine System

The cytokine network is made up of components that interact in a nonlinear fashion. It is thus feasible that complex behavior may occur even from few cytokine interactions if we look in the right place. In a recent study, TNFα levels in the aqueous humour (anterior chamber of the eye) after allogeneic corneal transplants were found to rise and fall repeatedly over many days (Chan et al., 1999). At first sight it is reasonable to assume that such complicated behavior must arise from very complex control mechanisms, but this may not be the case. To try and understand the dynamics of the response, a simple model of TNF regulation was proposed consisting of only effector cells producing TNFα, regulatory cells producing an inhibitor of TNFα, and an activation stimulus. When this model was mathematically analyzed, different types of behavior were observed that included steady state production of TNFα and oscillations depending on the strength of the allogeneic signal (Chan et al., 1999). Importantly, as the activation signal was increased, as would be found in allogeneic transplants, TNFα levels were found to fluctuate in a manner that was consistent with the experimental findings. This example shows that in a real biological (cytokine) system, a small number of simple but nonlinear interactions can give rise to very complex behavior.

Even such a simple model may be helpful in expanding our understanding of inflammatory disease. For example, spiking temperature during infection and other inflammatory diseases or the relapsing/remitting nature of some immunological disorders such as juvenile chronic arthritis (JCA) or familial Mediterranean fever (FMF) may be due to similar cytokine feedback regulation. Understanding the basis for the spiking TNFα production may also be important for predicting the best time to initiate therapy. It was also evident from this analysis that inadequate immunosuppression may make things worse by inducing high-level spiking production of TNFα. This aspect of control should be considered in the design of anti-TNF therapy for diseases such as cerebral malaria and rheumatoid arthritis.

Nonlinear Dynamical Analysis of the Cytokine Network

Our discussion so far has shown how nonlinear interactions may give rise to chaotic behavior in a simple cytokine system of four components (effector cells, regulatory cells, TNF, and an inhibitor). The cytokine network is of course much more complex than this and in general does not behave chaotically. We would not survive if it did. How then can this be studied? Clearly the cytokine network is far too complex to give a detailed analysis of its behavior in toto, but it is possible to use the tools of nonlinear dynamics to investigate in detail a subset of the whole network. This approach may allow in time a picture to be built up of how the network as a whole may function.

One of the most studied components of the cytokine network is the regulation of Th1 and Th2 T cell growth

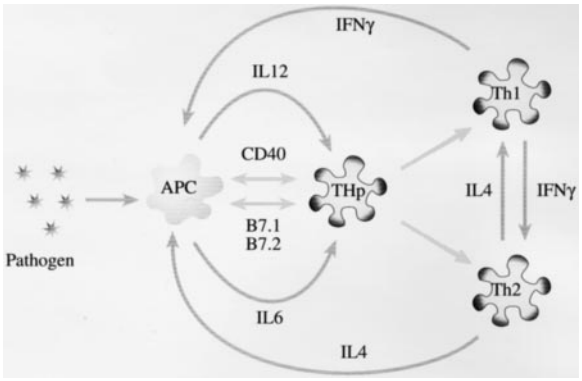

Figure 3. Cytokine Interactions Regulating Th1 and Th2 Differentiation

and differentiation. Th1 T cells are characterized by the profile of cytokines they secrete, namely, interleukin 2 (IL-2), TNFα, and interferon-γ (IFNγ), and are responsible for cell-mediated immune responses and inflammation (type 1 responses). Th2 T cells, on the other hand, secrete IL-4, IL-5, IL-10, and IL-13 and are required for optimal antibody production, particularly IgE, which is responsible for allergy (type 2 responses) (Abbas et al., 1996; Romagnani, 1997). A great deal of research has recently focused on the regulation of type 1 and 2 responses, because it has become clear that the wrong response can give rise to disease. For example, type 1 responses have been implicated in chronic inflammatory diseases such as rheumatoid arthritis, whereas type 2 responses may result in persistent bacterial (e.g., tuberculosis or leprosy) and parasitic (e.g., leishmaniasis) infections and are responsible for allergic disease. In these cases, therapeutic intervention that promotes switching from one type of response to another may enable a cure.

Following activation of precursor Th cells (THp) by antigen-presenting cells (APC) that have taken up antigen, the maturation and ultimate stability of the Th1 and Th2 T cell subsets is a function of the cytokines they themselves secrete. Thus, IL-4 is essential for the differentiation of precursor Thp cells into Th2 and at the same time inhibits production of proinflammatory cytokines by antigen-presenting cells and development of Th1 cells (Abbas et al., 1996; Romagnani, 1997). Similarly, IL-12 produced by APC and IFNγ produced by Th1 cells promote the maturation of Th1 cells and inhibit production of Th2 cells. A diagram indicating some of the main cytokine interactions and feedback loops involved is shown in Figure 3.

A nonlinear dynamical model of this system could take the form of a set of nonlinear differential equations describing interactions between the key components. These may include the cytokines IL-2, IL-4, IL-6, IL-12, and IFNγ. Each equation would describe the behavior of one component of the system as a function of the concentration of all the components. Numerical solutions to these equations give information about the behavior of the system as a whole. A detailed model has not been fully developed, but it is possible to show the form such a model might take. A simple model involving concentrations of Th1 and Th2 cells (or IFNγ and IL-4) would consist of a set of coupled nonlinear differential equations, with the variables representing concentrations of different cell types and signaling molecules (see legend for Figure 4). The functions f_1 and f_2 describe standard dose-response curves that are familiar to experimental immunologists and show the response of the system as a function of the concentration of one (or more) components (Figure 4).

Considerable information about the behavior of the system can be obtained from these models, even without rigorous mathematical solutions being found for the equations. One useful tool for this is the concept of state space. This is a graph where each axis represents one of the determining variables of the system. A point on the graph then represents any particular state of the system. Thus, a graph of the state space for Th1 and Th2 responses could have the concentration of Th1 cytokines on the x axis and that of Th2 on the y axis. On such a graph, it is possible to plot curves where there is no change in the concentration of Th1 or Th2 cytokines over time. Mathematically this is expressed by setting the differential equations shown in the legend for Figure 4 to zero: $d[\text{Th1}]/dt = 0$ and $d[\text{Th2}]/dt = 0$. These lines, termed the Th1 and Th2 nullclines, respectively, are shown in Figure 5A.

A great deal of information about the behavior of the system can be gained simply by inspection of these nullcline plots. For example, the points where nullclines intersect correspond to conditions where neither Th1 nor Th2 concentrations are changing over time and

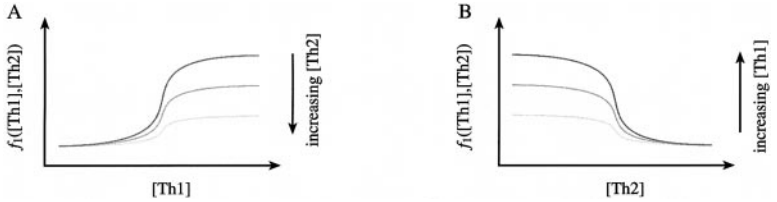

Figure 4. Dose-Response Curves for Th1 and Th2 Cytokines

The production of Th1 and Th2 cytokines would be expected to be dependent on the concentration of Th1 and Th2 cytokines as shown in these figures. Thus, the production of Th1 cytokines would be maximal at high concentrations of Th1 and low concentrations of Th2 (A), while the reverse would be seen for Th2 cytokines (B). These curves can be used to describe the behavior of Th1 and Th2 cytokines in the coupled differential equations.

$$\frac{d[\text{Th1}]}{dt} = a_1 + f_1([\text{Th1}],[\text{Th2}]) - \mu_1[\text{Th1}] \quad (1)$$

$$\frac{d[\text{Th2}]}{dt} = a_2 + f_2([\text{Th1}],[\text{Th2}]) - \mu_2[\text{Th2}] \quad (2)$$

where [Th1] and [Th2] are the concentrations of Th1 and Th2 cytokines, and a_1 *and* a_2 are the activation signals such as IL-12 delivered by the antigen-presenting cells that induce the Th1 and Th2 responses. The functions $f_1([\text{Th1}],[\text{Th2}])$ and $f_2([\text{Th1}],[\text{Th2}])$ describe the dose-response graphs for Th1 and Th2 shown in the figure. The terms μ_1*[Th1] and* $\mu_2[\text{Th2}]$ describe the clearance of Th1 and Th2 cytokines.

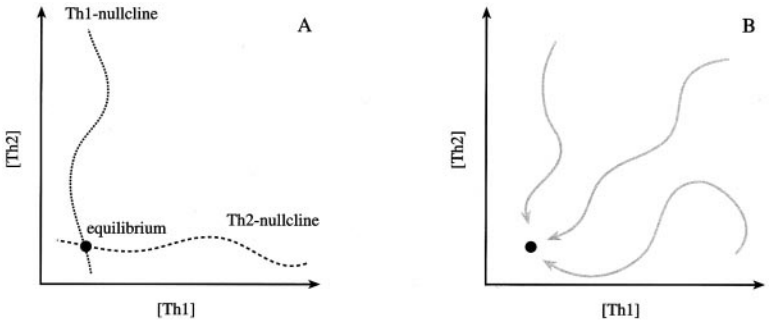

Figure 5. Nullclines with an Equlibrium Point, and Trajectories Converging to a Stable Equilibrium

Nullclines with a single equilibrium point (A) and the trajectories (B) for Th1 and Th2 dynamics in the absence of antigen stimulation.

hence the system is in equilibrium. Equilibria may be stable or unstable. In stable equilibria, small perturbations are transient and the system will move back to the equilibrium point. In unstable equilibria, small perturbations result in the system changing into another state. In the Th1 and Th2 example shown in Figure 5A, there is only one equilibrium point (where the concentrations of Th1 and Th2 do not change). In this case, it can be shown that the equilibrium is globally stable. This means that if you start at any other point in parameter space where the concentrations of Th1 and Th2 cytokines are not in equilibrium, the system will converge to the equilibrium point following a particular trajectory through the state space (Figure 5B).

Different sets of Th1 and Th2 nullclines can be obtained by consideration of the dose-response graphs shown in Figure 4 and the differential equations given in the legend. For further information about this process see for instance chapters 7 and 10 and appendix B of Othmer et al., 1997. Essentially three types of behavior are seen depending on the strength and type of activation signals delivered to the Th cells by antigen-presenting cells. The factors that determine the activation signal include antigen processing and presentation, production of cytokines such as IL-12, and expression of costimulatory molecules such as CD40, OX40, and CD80/86. The first type of behavior shown in Figure 5A occurs in the absence of significant activation for either Th1 or Th2. In this case there is a single equilibrium point. As discussed above, any perturbation of the system is transient and levels of Th1 and Th2 will always return to the equilibrium point where Th1 and Th2 responses are low or absent. This is exactly the behavior that we would intuitively expect in the absence of any antigenic stimulation.

Much more complex behavior can occur on antigenic stimulation. To illustrate this we will first consider a Th1 response in the absence of a Th2 response. If the Th1 dose-response curves shown in Figure 4 are shallow, increasing the activation signal for Th1 in the absence of an activation signal for Th2 will cause the equilibrium shown in Figure 5 to move smoothly to the right. This corresponds to a Th1 response in the absence of a Th2 response. If, however, the Th1 dose-response curves are steeper, as expected in a real life situation, we obtain a completely different nullcline pattern (Figure 6A), and the system behaves in a more complex manner. We now have three equilibria: two stable and one unstable (Figure 6A). In addition, a separatrix curve can be drawn to separate those initial conditions that converge to one stable equilibrium from those that converge to the other (Figure 6B). This means that depending on the exact initial conditions, the system may settle either to a low or high Th1 cell number. More significantly, as the activation signal for the Th1 response is gradually increased, the Th1 nullcline curve shown in Figure 6A will move upward, the two left-hand equilibria will suddenly vanish, and the system will converge to the only remaining equilibrium corresponding to a high Th1 concentration. If the system was previously at the left-hand stable equilibrium, it will suddenly jump to the right-hand equilibrium. This means that a very small increase in the Th1 activation signal can lead to a large increase in the Th1 response. Furthermore, as the activation signal is reduced, the Th1 response will not fall back immediately to the low Th1 equilibrium. In other words, the transitions from low to high and from high to low occur at different levels of activation signal. This is called hysteresis. Although we have only considered a Th1 response in the absence of a Th2 response, an identical argument will hold for a Th2 response in the absence of a Th1 response.

The pattern of behavior described for Th1 (or Th2) responses may have significant therapeutic implications. For example, if the high response represents Th1

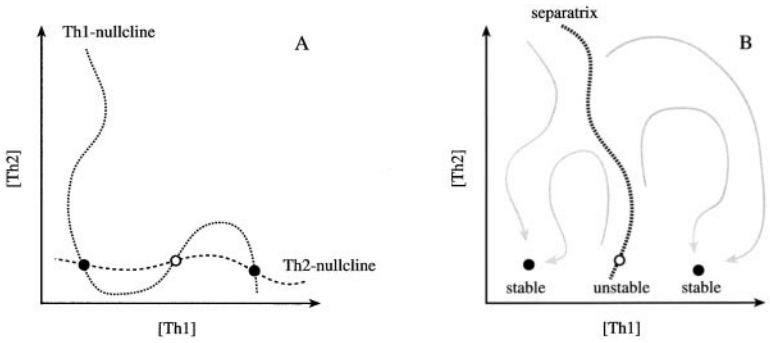

Figure 6. Nullclines with Equilibria and Trajectories for Intermediate Th1 Activation

Nullclines with stable and unstable equilibria (A) and trajectories (B) for Th1 and Th2 dynamics that result from Th1 but not Th2 activation.

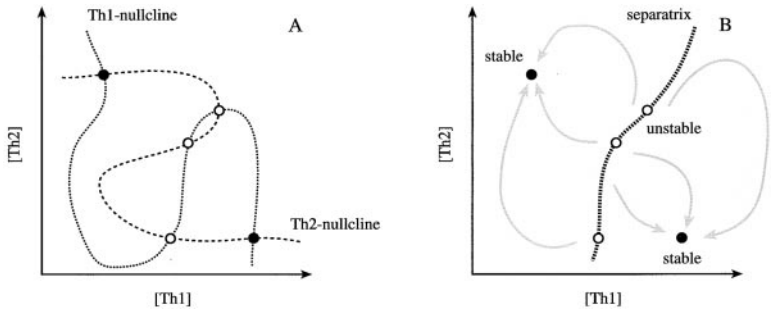

Figure 7. Nullclines with Equilibria and Trajectories for Intermediate Th1 and Th2 Activation

Nullclines with stable and unstable equilibria (A) and trajectories (B) for Th1 and Th2 dynamics that result from activation of both Th1 and Th2 responses.

cells in an inflammatory disease such as rheumatoid arthritis, decreasing the initial activation signal that induced the response may have no effect on the disease process until it can be reduced to a critical level well below that required to provoke the disease in the first place.

A third scenario to be considered is when activation signals for both Th1 and Th2 responses are present. This is perhaps the most common situation in the early stages of a T cell response, where cytokines and cell surface molecules involved in both Th1 and Th2 type activation can be present. In this setting a variety of complex behaviors is seen and examples of the possible nullclines, equilibria, and trajectories are shown in Figure 7. There are two stable equilibria, one corresponding to a Th1 response and the other to a Th2 response (the three other equlibria are unstable). The final state of the system (Th1 or Th2) will depend crucially on the starting conditions. Thus, the system is very dependent on the initial conditions, and small changes in the cytokine environment or the nature of the antigen presentation can have large consequences with respect to the final nature of the immune response. In addition we would expect hysteresis to be seen in the conversion of a Th1 equilibrium to a Th2 (or vice versa), which has clear implications for understanding the pathogenesis of diseases such as rheumatoid arthritis (Th1) and atopic dermatitis (Th2) and developing therapeutic strategies to convert one type of response to another.

General Properties of Nonlinear Networks

The nonlinear dynamical modeling of Th1 and Th2 T cell differentiation given above shows that the system can have properties that may not be obvious from biological/experimental considerations alone. Such complex nonlinear systems will commonly have properties that are not intuitive but which can have important biological implications. These include the following.

(1) It will not normally be possible to predict the behavior of the cytokine network even if all the major components are known and characterized. A good example of this property has been the inability in advance to predict the effect of cytokine or cytokine receptor gene inactivation experiments. Notable examples are the apparent immunological normality of IL-2 knockouts (Schorle et al., 1991) and the unexpected effect of LIF knockouts on embryo implantation with little effect on hematopoietic development (Stewart et al., 1992).

(2) Small changes in some components of nonlinear networks can have big effects whereas big changes may have little or no effect. This means that minor changes in cytokine concentrations or kinetics of production could in principle have major effects on the network as a whole or on a component of the network. An example described above is the TNFα gene dose effect, where wild-type mice with two functional *TNF*α genes produce up to 100 times more TNFα in response to LPS than heterozygotes with only one functional *TNF*α gene. Conversely, big changes in some components can have small effects such as the normal immune function in IL-2 knockout mice. This is likely to be very important for understanding the association between cytokine or cytokine receptor polymorphisms and disease.

(3) Some components of nonlinear networks will be essential for stability of the network as a whole and/or existence of an attractor whereas others may not be required. For example, mutations in the IL-2Rγ chain result in a severe T cell and B cell immunodeficiency, whereas IL-4 seems only to be required for IgE production. The latter is often called cytokine redundancy; that is, cytokines with apparently similar activities can replace the function of one another. Pairs of cytokines with similar activities include IL-1α and IL-1β, IL-4 and IL-13, and IL-2 and IL-15. However, these pairs of cytokines do not occupy the same position in the network, and it is more likely that the network as a whole can remain stable in the absence of some components than that one cytokine can cover for the absence of another.

(4) Perhaps one of the most interesting and unexpected properties of complex nonlinear networks is their tendency to occupy a limited number of stable states out of the theoretically huge numbers of states available to them. These attractors often have great stability. In the example given above, these attractors may be responsible for Th1 and Th2 differentiation.

(5) Finally, both regular and irregular oscillations are often found in complex networks. These may be responsible for properties such as diurnal rhythms in cytokine levels and febrile cycles in inflammatory or infectious diseases and for other recurrent illnesses such as familial Mediterranean fever or juvenile chronic arthritis.

Final Word

Mathematical models will not replace laboratory experimentation, but they do have an important complementary role for understanding the complexities of the cytokine network and other biological systems. Such models cannot incorporate every component of the network, as the model itself would then be impossible to analyze. Rather, they should be considered as virtual laboratories

in which numerical experiments can be performed that could not otherwise be undertaken. Such virtual experiments can deal with more parameters than in vitro or in vivo experimentation and can give insights not available through more conventional investigations. A dialogue between the two disciplines is required. The biologists should define the problem and provide quantitative information about the system from which a model can be constructed. Mathematical analysis of the model can then make predictions about the behavior of the biological system that can be tested in the laboratory. The more refined data obtained from these experiments can then be fed back into the model. By this iterative process, mathematics and biology can combine to give a deeper understanding of the cytokine network and other biological processes.

References

Abbas, A.K., Murphy, K.M., and Sher, A., (1996). Functional diversity of helper T lymphocytes. Nature *383*, 787–793.

Amiot, F., Boussadia, O., Cases, S., Fitting, C., Lebastard, M., Cavaillon, J.-M., Milon, G., and Dautry F. (1997). Mice heterozygous for a deletion in the tumour necrosis factor alpha and lymphotxin alpha genes: biological importance of a non-linear response of tumour necrosis factor alpha gene dosage. Eur. J. Immunol. *27*, 1035–1042.

Barnes, K.C., and Marsh, D.G. (1998). The genetics and complexity of allergy and asthma. Immunol. Today *19*, 325–332.

Chan, C.C.W., Stark, J., and George, A.J.T. (1999). Analysis of cytokine network dynamics in corneal allograft rejection. Proc. R. Soc. Lond. B., in press.

Hobbs, K., Negri, J., Klinnert, M., Rosenwasser, L.J., and Borish, L. (1998). Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. Am. J. Respir. Crit. Care Med. *158*, 1958–1962.

Hurme, M., Lahdenpohja, N., and Santtila, S. (1998). Gene polymorphisms of interleukins 1 and 10 in infectious and autoimmune diseases. Ann. Med. *30*, 469–473.

Mullighan, C.G., Fanning, G.C., Chapel, H.M., and Welsh, K.I. (1997) TNF and lymphotoxin-α polymorphisms associated with common variable immunodeficiency: role in the pathogenesis of granulomatous disease. J. Immunol. *159*, 6236–6241.

Othmer, H.G., Adler, F.R., Lewis, M.A., and Dallon, J.C. (1997). Case Studies in Mathematical Modelling. (New Jersey: Prentice Hall).

Romagnani, S. (1997). The Th1/Th2 paradigm. Immunol. Today *18*, 263–268.

Schorle, H., Holtschke, T., Hunig, T., Schimpl, A., and Horak, I. (1991). Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. Nature *352*, 621–624.

Shinbrot, T., Grebogi, C., Ott, E., and York, J.A. (1993). Using small perturbations to control chaos. Nature *363*, 411–417.

Stewart, C.L., Kaspar, P., Brunet, L.J., Bhatt, H., Gadi, I., Kontgen, F., and Abbondanzo, S.J. (1992). Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature *359*, 76–79.

Turner, D., Grant, S.C., Yonan, N., Sheldon, S., Dyer, P.A., Sinnott, P.J., and Hutchinson, I.V. (1997). Cytokine gene polymorphism and heart transplant rejection. Transplantation *64*, 776–779.

Woo, P. (1997). The cytokine network in juvenile chronic arthritis. Pediatr. Rheumatol. *23*, 491–500.